

MarA Inhibitors

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During the process of infection a pathogen must first invade a host and then attach itself to a tissue. Once inside the host a pathogen may express toxins or other host avoidance proteins and is under constant pressure to respond to changes in pH, temperature, osmolarity, and the presence of antibiotics. These events are primarily controlled at the transcriptional level by regulatory elements such as the multiple adaptational response (Mar) system of *E. coli*. The Mar system is a regulatory locus that encodes a transcription activator, termed MarA, which is a member of the AraC family of DNA binding proteins. Proteins within this highly conserved family are characterized by a dual HTH DNA-binding motif. There are two subfamilies of AraC proteins, the small (~15kDa) group such as MarA and SoxS which only possess a DNA binding domain, and the larger proteins (~30kDa) such as AraC, Rob, ToxT, BfpT, and ExsA that possess the DNA binding domain and an additional domain with another function. Over 1,000 orthologs of AraC proteins have been identified in gram-negative pathogens such as *E. coli* (UPEC and EPEC), *Shigella*, *Salmonella*, *Yersinia*, *Proteus*, *P. aeruginosa*, *Enterobacter*, *Klebsiella*, *V. cholerae*, *N. gonorrhoea*, gram-positive organisms, including *S. aureus*, *Streptococcus*, *Enterococcus*, *Bacillus*, and the mycobacteria.

Experiments in a murine pyelonephritis model of infection using a multidrug resistant *E. coli* strain illustrate the importance of MarA, SoxS, and Rob in virulence. Infection with the wild type organism persisted for at least 11 days. However, when *marA*, *soxS*, and *rob* were deleted, the infection was cleared within 5 days. Bieber, et al reported that in EPEC, deletions in PerA severely attenuated infection in humans (Science 1998, **280**:2114). In *V. cholerae*, ToxT plays a significant role in the infant mouse model, with mutants of either El Tor or classical biotype strains showing significantly decreased lethality at 24 hours (Champion et al, 1997 Mol Micro **23**:223). LcrF, a MarA analog from *Yersinia*, controls expression of a type III secretion system, which injects cytotoxic Yops into macrophages and other host cells. Some Yops, like YopJ inhibit the MAPK cascade while others including YopE disrupt actin in microfilament formation and block phagocytosis and inhibit the oxidative burst like YopH.

The function of MarA is to regulate a number of cellular functions including multi-drug resistance, metabolism, DNA repair, biofilm formation, cell envelope synthesis, tolerance of organic solvents, virulence, and transport. The crystal structure of MarA has been solved (Rhee et al PNAS **95**:10413 and Kwon et al 2000 Nature Struct Biol. **7**:424.) and the interactions of MarA and Rob with DNA have been defined.

Many small-molecular weight MarA inhibitors have been identified. These agents inhibit protein-DNA interactions in vitro and have shown activity against whole bacterial cells in virulence assays. Multiple compounds have been tested against *P. aeruginosa* (ExsA), *Y. pseudotuberculosis* (LcrF), and *E. coli* (SoxS). Several exhibit efficacy in an *E. coli* pyelonephritis and *Yersinia* lung infection models. In the former, mean bacterial counts in the kidney were reduced by over 3.5 logs when an exemplary compound was administered at 1mg/kg over a period of five days. Dose escalation studies with this agent showed a significant (4-log) reduction in bacteria recovered from the kidney.

Future plans for developing MarA inhibitors will exploit a non-antibacterial approach for infectious disease therapy that will hopefully avoid the traditional drivers for the development of antibacterial resistance. Since MarA and its relatives are well-conserved targets, the opportunity for developing therapy against a variety of agents, particularly *Yersinia*, *E. coli*, *Pseudomonas*, and *Vibrio*, is envisioned. Presently, efficacy in vivo has been shown for *E. coli* and *Yersinia*, and future studies are expected in order to develop screening systems for other agents.

The research needs identified by these studies include the full range of preclinical development and clinical support. Large pharmaceutical companies have deserted this exciting field, just as new opportunities are emerging.